CHAPTER 8

Bioreactors in Tissue Engineering

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Summary

Despite the early promise of tissue engineering, researchers have faced challenges in regenerating tissues that serve a predominantly mechanical function. Current approaches investigate the use of bioactive or bioresorbable matrices, which rely on the appropriate cellular response in vivo, with the intention of developing biological and physical functionality after implantation. A limitation of this approach is the variability of the patient response in terms of resorption, recellularisation and regeneration, which can result in development of inappropriate implant properties. A step further is the utilisation of passive in vitro recellularisation prior to implantation. The success of this approach is also limited since cell differentiation and tissue remodelling do not progress physiologically. Functional tissue engineering is a more promising approach that employs appropriate in vitro-propagated cells to cellularise scaffolds, coupled with appropriate physical conditioning, with a view to establishing tissue functionality prior to implantation. Studies have produced considerable evidence suggesting that physical stimuli may affect gene expression and significantly increase the biosynthetic activity in a range of different cells. The fact that physical stimuli can modulate cell function has motivated the development of functional simulations systems to recellularise tissues in vitro by exposing them to physical stimuli. The development of such technologies will not only provide tissue engineering solutions, but will also provide important in vitro model systems for the enhancement of

understanding into mechanotransduction. This review focuses on how mechanotransduction dictates cell function, as well as on the bioreactor systems that have been developed to investigate this phenomenon.

Keywords: cell signalling, cell adhesion molecules, mechanotransduction, bioreactors

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Introduction

Conventional therapies for repair of tissues most commonly use synthetic biomaterials or chemically cross-linked xenograft tissue. Both these approaches only deliver inert or biocompatible material solutions that cannot develop or grow with the patient and may calcify, become rigid and degenerate. Ideally, surgeons would prefer autologous tissue for this type of repair since it will retain viability and regenerate. In most cases, however, autologous tissue is not available. Tissue engineering offers an attractive alternative involving the development of biological or hybrid substitutes for implantation into the body with the purpose of fostering remodelling and regeneration of diseased tissue. Tissue engineering follows the principles of cell transplantation, materials science, and bioengineering towards the development of substitutes that would restore and maintain normal function.

Over the years, three principal tissue engineering approaches have been researched for treating diseased or injured tissues. These include (i) direct implantation of freshly isolated or cultured cells; (ii) in situ tissue regeneration; and (iii) implantation of tissues assembled *in vitro* from cells and scaffolds. Direct cell implantation involves isolating individual cells or small cellular aggregates from the recipient or a donor, which are expanded in culture and injected into the damaged tissue directly. In situ tissue regeneration involves the use of bioactive and/or bioresorbable natural or synthetic scaffolds to exploit the body's natural ability to regenerate. This approach has been investigated for repairing ligaments and tendons (1), heart valves, bladder (2), small-diameter vascular grafts (3, 4) and surgical patches (5). However, the major limitation of this approach is the variability of the recipient response in terms of resorption, recellularisation and regeneration, which can result in development of inappropriate mechanical and biological properties of the implant *in vivo* and consequent failure. A step further is the utilisation of passive *in vitro* recellularisation of appropriate scaffolds prior to implantation. While such an approach has been attempted (6), its success is

limited since cell differentiation and matrix remodelling do not progress in the physiological manner (7, 8).

Functional tissue engineering

Despite the early success of tissue engineering, workers in the field have faced challenges in repairing or replacing tissues that serve a predominantly mechanical function. Two potential obstacles to the creation of functional tissues that will be integrated into the host are inadequate mechanical properties (e.g. ability to withstand haemodynamic stresses) and adverse host-tissue reactions due to immunogenicity of the cellular components or the presence of residual non-degraded polymer scaffold. The requirement is that the implant delivers biological and physical functionality upon implantation, carries a negligible safety risk in the form of a low immunogenic or tumorigenic potential, and retains its capacity for self repair.

An evolving discipline called functional tissue engineering (FTE) seeks to address the obstacles associated with conventional tissue engineering approaches. The aim of FTE is to grow a complete three-dimensional tissue *in vitro* and then implant it once it has reached "maturity". This scientifically challenging approach employs appropriate *in vitro*-propagated and -manipulated autologous cells to cellularise three-dimensional scaffolds, coupled with appropriate physical conditioning of the tissue *in vitro*, with a view to produce tissue functionality prior to implantation. Although this technique will also rely on the body's ability to regenerate, additional cues would be provided with the presence of functional tissue at the time of implantation. As compared to *in vivo* transplantation of dissociated cells and/or biodegradable scaffolds alone, the implantation of a functional engineered tissue has the potential to improve the localization of cell delivery and promote graft fixation and survival (9).

Based on the principle of FTE, there is now considerable interest in developing the appropriate physical environments *in vitro* using biomechanically active simulations systems, known as bioreactors, to recellularise tissues *in vitro* in a functional manner (10-12). Such an approach to tissue engineering has the potential to provide not only an essentially unlimited pool of transplants, but also better means to control quantitatively the cell culturing parameters that lead to appropriate tissue development *in vitro* (13). Bioreactors can be designed to maintain physiological parameters at desired levels, enhance mass transport rates, and expose cell-seeded three-dimensional scaffolds to specific biochemical or physical stimuli (9). The ingredients of FTE include i) cells capable of differentiating into the appropriate lineages, ii) a scaffold that provides a structural template for tissue development, and iii) a bioreactor that guides appropriate cell differentiation and tissue development by providing the necessary biochemical and physical regulatory signals (13).

Regulation of cell function in tissue engineering

Currently, the regulation of cell function and the growth of three-dimensional tissues are major areas of focus in developing new tissue engineering techniques. In order to determine design criteria for tissue engineering, it is necessary to understand how complex physiological pathways function within the physical context of cells and tissues. For a cell to respond to its environment extracellular signals need to be sensed, reach the nucleus, and then have to trigger the specific expression/repression of particular genes. These extracellular signals can promote or restrain cell proliferation, migration and differentiation, trigger matrix remodelling, or promote enhanced tissue organization. Understanding how to manipulate signalling through adhesion receptors to promote the desired end-points for specific tissue engineering problems is a critical key to successful tissue repair and reconstruction (14). The growth and differentiation of many cell types, and subsequently, tissue patterning and architecture, is regulated by

four major sources of external signalling (Fig. 1). These include: i) soluble growth and differentiation factors (15-17), ii) nature and organization of insoluble and soluble extracellular matrix (ECM) constituents (14, 18-20), iii) intercellular interactions (14), and iv) environmental stress induced by fluid flow and/or mechanical stimuli such as dynamic, static or shear forces, as well as other physical cues such as oxygen tension and pH effects (13, 21, 22). These stimuli applied individually or in combination can have a dramatic impact on the tissue growth, and can be used to modulate cell commitment and differentiation, recapitulating the events occurring *in vivo* during tissue development.

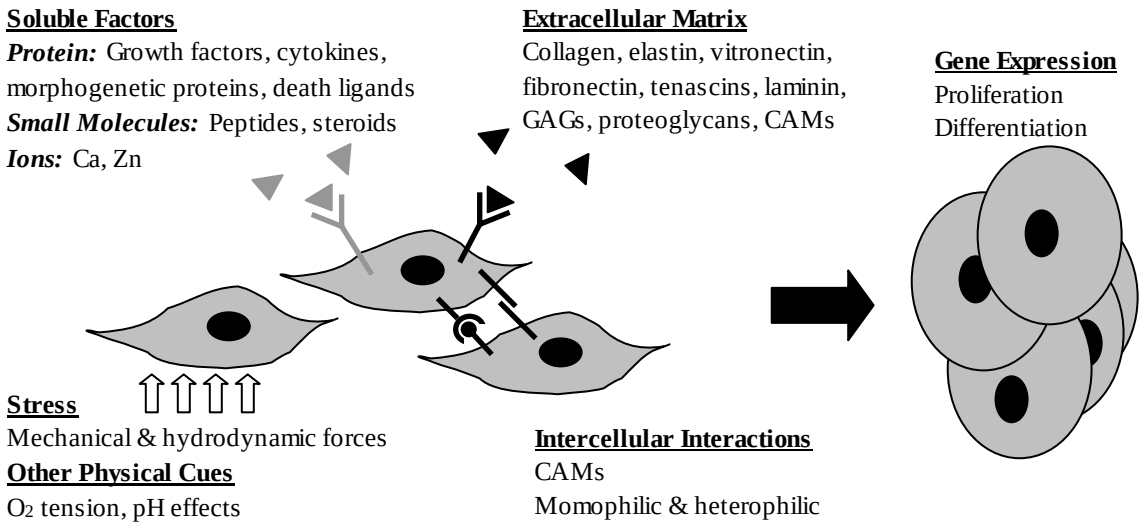

Fig. 1: Modulation of cell fate by extrinsic factors.

Soluble factors include cytokines, growth factors, morphogenetic proteins, small-molecule agonists, steroid hormones and ions. These factors feed into complex interacting networks of signal transduction pathways. Regulation of cell growth versus differentiation requires appropriate combinations of factors, whereas *in vitro* studies have shown that several growth factors present biphasic dose-response characteristics. This suggests that excessive amounts may have diminished or inappropriate biological impact on cell function. Insoluble and soluble ECM components include collagenous proteins, non-collagenous proteins, as well as proteoglycans and glycosaminoglycans (GAGs). The dynamic composition of the ECM acts as a reservoir for signalling molecules and mediates signals from other sources to the cells during adhesion, migration, proliferation, and differentiation. In order to regulate appropriate cell function, scaffolds for *in vitro* cell seeding have to mimic the natural ECM by presenting architectural and compositional properties that guide cell activity towards the synthesis of appropriate new tissue (23). In addition, they have to possess appropriate surface properties and to contribute the necessary mechanical properties of the cell/scaffold composite (24-27). Tissue engineering scaffolds may consist of natural or synthetic polymers or a combination of both. The presence of collagen, elastin and GAGs in the majority of human tissues, and their ability to support the function of a wide variety of cell types, makes natural polymers the most widely used scaffold constituents in tissue engineering (1, 28-33).

If groups of cells are to combine together to form part of an organ or tissue it is imperative that each cell is in its proper place and is able to communicate with other cells. Interactions between cells and with the ECM are largely involved in creating the structure of a tissue, but may also facilitate cell-cell communications. Cell-cell and cell-ECM interactions are communicated by adhesion receptors, which control both anchorage and molecular signal transduction. These receptors promote or restrain cell proliferation, differentiation, and cell death, trigger matrix remodelling and determine tissue organization. A key feature of their function is their ability to organize signalling complexes at sites of contact with their extracellular environment. The manipulation of

cell-cell and cell-ECM interactions is the key to promoting the desired end-points for tissue engineering strategies (21, 22).

Cells bind via specific receptors to the ECM, which provides elasticity and resistance to mechanical forces. However, in tissues such as epithelia, where the only point of contact with the ECM is through the basement membrane, the cell-cell interactions are adapted to bear tensile and compressive stresses. Cell junctions are formed by specialised molecules known as cell adhesion molecules or CAMs, which promote selectivity, diversity and complexity in cell/tissue functions, as well as structural organization. CAMs are classified into families dependent upon their generalised structure. Accordingly, there a five families of "classic" CAMs, including cadherins, integrins, selectins, proteoglycans (syndecans), and the immunoglobulin superfamily of adhesion molecules. These CAMs, together with their associated role in cell function, are illustrated in Table 1.

CAM Family	***Cytoskeletal Association***	***Cell Function***
Cadherins	Actin filaments	Adhesion belt Desmosomes
Immunoglobulin superfamily	Intermediate filaments (some)	
Selectins	None	Cell homing
Integrins	Actin filaments Intermediate filaments	Focal contacts Hemidesmosomes
Proteoglycans	None	Binding and regulation of cytokine and enzyme activity

Table1. Families of cell adhesion molecules (CAMs).

Cadherins and integrins are the principal CAMs involved in holding cells/tissues together and mediating cell-cell and cell-ECM interactions, respectively. Adhesion and signalling between cells and the ECM are frequently mediated by receptor proteins of the integrin family (34). These adhesion molecules are particularly relevant to wound healing, cell shape, cell differentiation, apoptosis, and trafficking of cells to different tissue compartments. Integrin-ligand interactions are accompanied by clustering and activation of the integrins on the cell surface, which is also accompanied by the transduction of signals into intracellular signal transduction pathways that mediate a number of intracellular events. Signals transmitted internally can lead to changes in cell motility, DNA transcription, and enhanced differentiation (35). Signals can also be transduced from the cell interior to the cell surface. This leads to the "activation" of integrins which become capable of binding (34). Integrins allow fibroblasts, other mesenchymal cells and white cells to adhere to fibronectin and collagen as they move through the ECM. Integrins bind epithelial and muscle cells to laminin in the basement membrane, providing the physical attachments necessary to transmit internal forces to the ECM and resist external forces. These interactions are essential for the tissue development and integrity and thus their expression and binding is essential to tissue engineering principles. Hence, including ECM matrix ligands for cells in tissue constructs is essential, and is of particular relevance to tissue engineering using synthetic matrix biomaterials that do not normally express integrin ligands. It is essential to supplement such matrices with appropriate cell-adhesion-promoting ligands such as peptide sequences (RGD) to gain optimal cell function.

Several studies have indicated that there is high interdependency between cell shape and morphology, cell-cycle progression and cell differentiation. Signals transmitted by ECM receptors affect cell-cycle progression by modulating the activity of certain enzymes (kinases). ECM-mediated changes in cell shape lead to alterations in the cell ability to undergo ligand-dependent proliferation. The dynamic state of a cell culture, where adhesive connections between cells and between cells and the ECM are being

made and broken, can lead to cellular responses that are not observed under static conditions (36, 37). The proper function of many cells grown *in vitro* is highly dependent on their state of differentiation, which is defined by changes in the gene expression profile. It is important to note that the gene expression patterns induced by three-dimensional cultures have been shown to be distinctly different to those found in two-dimensional monolayer cultures, suggesting that the three-dimensional architecture of the ECM profoundly influences the state of differentiation of many cell types cultured *in vitro* (24).

The role of mechanotransduction in tissue engineering

The term mechanotransduction refers to the process by which the cells transduce mechanical stresses into biochemical signals to regulate their function. Cells in tissues constantly experience mechanical stimuli. Even cells in static culture experience the effects of gravity. Physical stimuli such as shear-stress, fluid-flow, compression and tension, not only alter the organization and distribution of structural elements and organelles within cells, but also become transduced into biochemical inputs that modulate signalling networks within and between cells. Mechanical stress is an important modulator of cell physiology and it is believed that the intracellular mechanical environment is important in tissue homeostasis. There is considerable evidence that physical stimuli affect gene expression and significantly increase the biosynthetic activity in a range of different cell types (21). The effects of the mechanical environment on the behaviour of bone cells has been extensively studied over the years (23, 38-41), and more recently evidence has accumulated that stromal cells in non-skeletal tissues also respond to mechanical stimuli. In fact, surgeons already make use of mechanical forces to achieve desired *in vivo* responses, such as distraction osteogenesis and skin expansion.

Extensive research on the effect of mechanical stimuli on cell metabolism has suggested that tissues may respond to mechanical stimulation via loading-induced flow of the interstitial fluids. Studies have also shown that integrins mediate transmembrane transfer of mechanical signals and that some forms of mechanochemical transduction occur within the specialized cytoskeletal complex known as the focal adhesion that forms at the site of integrin binding to the ECM. Integrin stimulation by mechanical stress can activate specific signalling pathways. Forces transmitted bi-directionally between the cytoskeleton and the ECM influence gene expression and, subsequently, protein synthesis, cell proliferation and differentiation (42). Moreover, mechanical stresses that produce cell distortion can also switch cells between growth, differentiation, motility, and apoptosis programs. It has been shown that synergistic cues of integrin-mediated mechanotransduction and cell shape cause round cells to become apoptotic and spread cells to express a growth response (22).

Recent studies have postulated that culturing cells in the appropriate biochemical environment and in the presence of the mechanical stimuli that the cells encounter *in vivo*, could provide the correct signals for cellular differentiation and production of the desired ECM with appropriate physical properties. For example, cell-ECM interactions have been shown to switch hepatocytes between growth and differentiation (20), and mechanically stressed dermal fibroblasts differentiate into myofibroblasts (43). Moreover, fluid-induced shear stress has a well-known impact on vascular cell morphology, proliferation, and orientation. Exposure of monolayer bovine articular chondrocytes to fluid flow has been shown to activate the MEK1/ERK signalling pathways. This activation ultimately leads to transcriptional down-regulation of aggrecan gene expression (44). Schwachtgen *et al.* (45) showed the transcriptional activation of the Egr-1 gene in endothelial cells in response to fluid flow requires signalling through the ERK1/2 pathway. Moreover, it has been shown that the long term application of cyclic strain *in vitro* increases the organisation of the ECM, leading to improved mechanical properties of engineered smooth muscle tissue(11). Exposing cartilage constructs to dynamic compression at physiological frequencies has been

shown to enhance ECM synthesis rates (46). The concept that tensile loading is important in tissue development has been extended by exploiting the native contractile properties of collagenous cell culture substances (47), and in the tissue engineering of tendon and ligament (13, 48). Studies have also shown that mechanically-stressed bladder smooth muscle cells are acutely receptive to their mechanical environment and cyclical deformation induces connective tissue synthesis (49-51).

Tissue engineering bioreactors

The term "bioreactor" refers to a system in which conditions are closely controlled to permit or induce certain behaviour in living cells or tissues. The concept of bioreactors is neither new nor restricted to tissue engineering. Microbiologists use bioreactors (chemostats) to grow cultures of microorganisms under defined conditions. Bioreactors are also used in the brewing, food, pharmaceutical and biotechnology industries. The fact that physical stimuli can modulate cell function and tissue development has motivated the development of biomechanically active simulation systems to recellularise tissues *in vitro* by exposing them to physiologically relevant mechanical and/or hydrodynamic stimulation. Bioreactor technologies intended for tissue engineering can be used to grow functional cells and tissues for transplantation, and for controlled *in vitro* studies on the regulation effect of biochemical and biomechanical factors on cell and tissue development. The primary objectives of these systems are to establish spatially uniform cell distributions on three dimensional scaffolds, to maintain desired concentrations of gases and nutrients in the culture medium, and to expose developing tissue to appropriate physical stimuli.

The requirements for a FTE bioreactor will vary depending on the dimensions, complexity, and physiological environment of the tissue to be engineered. The overall goal is to have systems that reliably and reproducibly form, store, and deliver functional

tissues that can sustain function *in vivo*. In essence, the bioreactor needs to provide the appropriate physical stimulation to cells, continuous supply of nutrients (e.g. glucose, amino acids), biochemical factors and oxygen, diffusion of chemical species to the construct interior, as well as continuous removal of by-products of cellular metabolism (e.g. lactic acid). Moreover, such a bioreactor has to be able to operate over long periods of time under aseptic conditions since maturation of a functional tissue may take up to 3-4 months. Providing three-dimensional tissues with nutrients may rely on passive diffusion, or may be more actively delivered by direct perfusion. However, direct perfusion introduces a new level of complexity when scale-up is encountered, and the engineering challenges may be significant. Tissues that have been manufactured to date have relied on diffusion, although tissues envisioned for future products will require a more active delivery process.

Another important issue in the design of FTE bioreactors is the monitoring of tissue growth. Minimising variability of growth conditions does not necessarily result in perfectly uniform growth between batches and, therefore, it is necessary to monitor growth during culture to ensure that the harvest time is optimal for each batch. The monitoring method is likely to be individualised for each tissue, although the monitoring of glucose uptake has been used successfully in the tissue engineering of different tissues. Nevertheless, for tissues that serve a predominantly mechanical function, monitoring the mechanical properties during tissue growth may be also necessary. An advantage in the area would be the development and employment of techniques which could test the integrity of the tissue non-invasively. This would allow for a higher efficiency in the tissue engineering process, as well as a high degree of certainty in harvesting tissues within the pre-determined manufacturing specifications.

Over the past few years several systems have been employed to induce different types of physical stimulation to cells *in vitro*. Simple systems include simple dishes, spinner flasks and rotating vessels in which tissue matrices are fixed or floating and the culture medium is exchanged batch-wise at appropriate intervals (Fig. 2). Other designs are

based on perfused columns or chambers in which the tissue matrices are fixed and there is continuous medium recirculation (Fig. 3). In these systems, physical conditioning of the tissue-engineered constructs relies upon hydrodynamic shear forces. Engineered cartilage grown in mixed flasks has been shown to be structurally superior to that grown in orbitally mixed dishes, which was in turn superior to that grown statically (52). It has been hypothesised that hydrodynamic forces affect cultured cells via pressure fluctuations that stretch the cell membranes, and/or through shear stress (53). Such bioreactors have been shown to support the growth of tissue up to a maximum thickness of 5 mm (9). Bioreactor systems that expose growing tissues to dynamic tension (47, 48, 54, 55), compression (46) or hydrodynamic pressure (56) have also been described. In these systems the presence of mechanical forces during cultivation stimulated tissue development by providing stimuli at physiological frequencies and loading.

Fig. 2: a) Spinner flask bioreactor, b) Synthecon ® rotating wall vessel bioreactor.

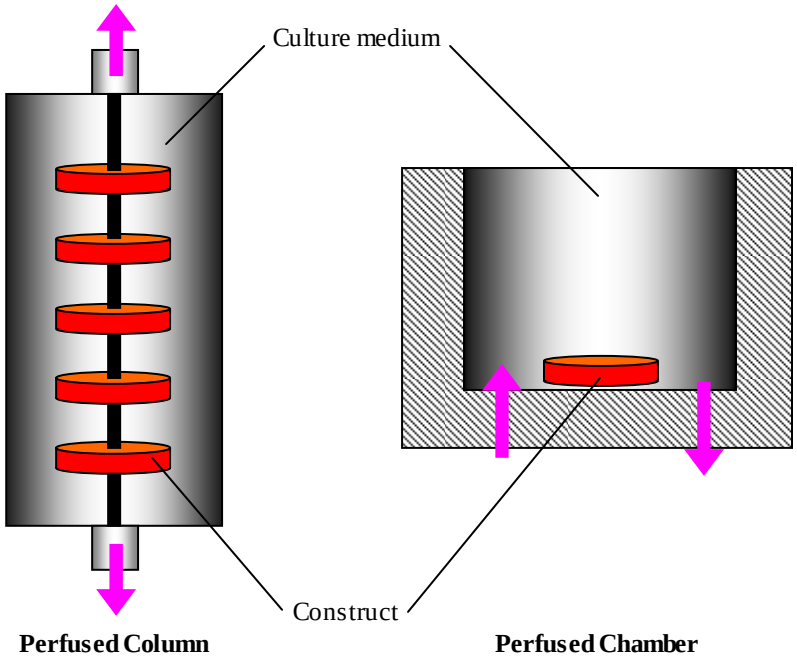

Fig. 3: Perfused bioreactor systems.

In addition to bioreactor systems that provide either hydrodynamic stimuli through continuous perfusion or mechanical stimuli alone, several groups have demonstrated the advantages of using culture systems that combine both hydrodynamic and mechanical stimulation during tissue development (57-60) (Figs. 4 and 5). Niklason *et al.* (61) were able to fabricate solid vascular tissue comparable to a native vessel using smooth muscle cell-seeded tubular polymer scaffolds cultivated in the presence of pulsatile radial stress. Sodian *et al.* (62) have developed a pulsatile bioreactor for the fabrication of tissue engineered surgical patches. Zeltinger *et al.* (12) have developed a novel bioreactor that imposes dynamic pulsatile flow to fibroblast-seeded decellularised

porcine aortic valves with a view to tissue engineering a functional aortic valve for implantation. In all the above studies, perfusion of culture medium improved tissue growth and metabolism by enhancing mass transfer and reducing the variations in the concentrations of gases, nutrients, metabolites, and regulatory factors that occur in periodically re-fed cultures.

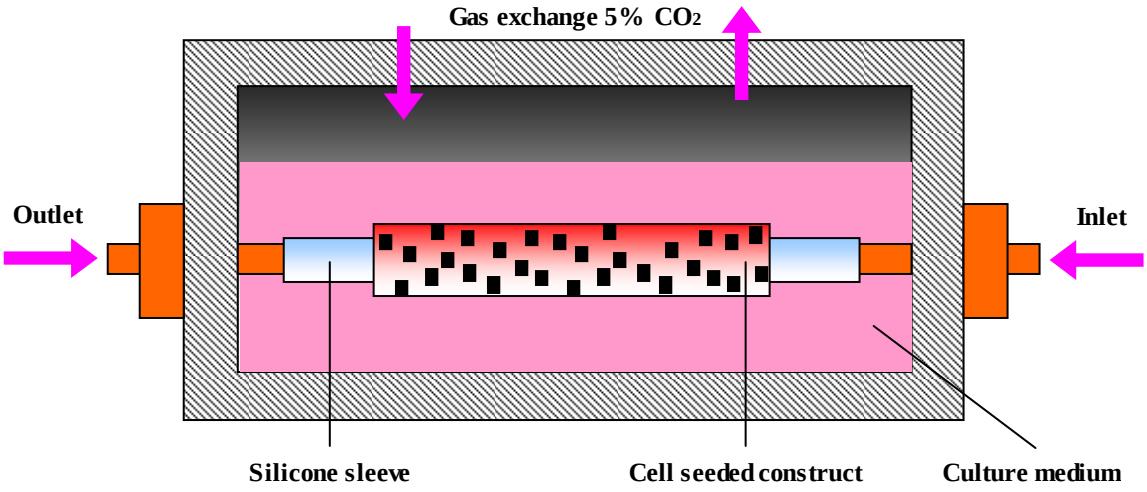

Fig. 4: Bioreactor system for vessel tissue engineering.

Fig. 5: The Leeds six-station pulsatile flow heart valve bioreactor.

Concluding remarks

Although the molecular mechanisms underlying the effect of mechanical stimulation on cell function and tissue development have yet to be determined, the basis of mechanotransduction responses is beginning to be unravelled (63). The majority of studies, however, have been carried out in simple two-dimensional culture systems in which the cells are subjected to fluid flow and/or stretch. Most cell types *in vivo* exist in three-dimensional systems and there are as yet, few studies of mechanotransduction in three-dimensional systems. Moreover, the type and appropriate amount of physical stimuli needed to improve tissue formation remains speculative. Factors such as level and direction of mechanical strain, dynamic versus static force regimens, as well as oscillation frequency, amplitude, and cycle form may be critical for the tissue remodelling response. Therefore, the development of functional simulation systems for the growth of tissues in response to mechanical stimulation will not only provide tissue engineering solutions but will also provide important *in vitro* model systems for the enhancement of understanding into mechanotransduction, and the relationship between physical conditions, cellular function, tissue development and tissue properties, underpinning the development of tissueomics research.

References

1. Kato YP, Dunn MG, Zawadsky JP, Tria AJ, Silver FH. Regeneration of Achilles tendon with a collagen tendon prosthesis. Results of a one-year implantation study. J Bone Joint Surg Am 1991; 73:561-574.
2. Kambic H, Kay R, Chen JF. Biodegradable pericardial implants for bladder augmentation: a 2.5 year study in dogs. J Urol 1992; 148:539-543.

3. Ferrand BK, Kokini K, Badylak SF, Geddes LA, Hiles MC, Morff RJ. Directional porosity of porcine small-intestinal submucosa. J Biomed Mater Res 1993; 27:1235-1241.
4. Conklin BS, Richter ER, Kreutziger KL, Zhong DS, Chen C. Development and evaluation of a novel decellularized vascular xenograft. Med Eng Phys 2002; 24:173-183.
5. Del Nido PJ, Williams WG, Wilson GJ, Coles JG, Moes CA, Hosokawa Y, McLaughlin PR, Fowler RS, Izukawa T, Rowe RD. Synthetic patch angioplasty for repair of coarctation of the aorta: experience with aneurysm formation. Circulation 1986; 74 (3 Pt 2):I32-36.
6. Berglund JD, Mohseni MM, Nerem RM, Sambanis A. A biological hybrid model for collagen-based tissue engineered vascular constructs. Biomaterials 2003; 24:1241-1254.
7. Woo SL, Hildebrand K, Watanabe N, Fenwick JA, Papageorgiou CD, Wang JH. Tissue engineering of ligament and tendon healing. Clin Orthop 1999; (367 Suppl):S312-323.
8. Butler DL, Awad HA. Perspectives on cell and collagen composites for tendon repair. Clin Orthop 1999; (367 Suppl):S324-332.
9. Freed LE, Vunjak-Novakovic G. Tissue Engineering Bioreactors. Lanza RP, Langer RP, Vacanti J, editors. Principles of tissue engineering. New York: Academic Press; 2000:143-154.
10. Sodian R, Lemke T, Fritsche C, Hoerstrup SP, Fu P, Potapov EV, Hausmann H, Hetzer R. Tissue-engineering bioreactors: a new combined cell-seeding and perfusion system for vascular tissue engineering. Tissue Eng 2002; 8:863-870.
11. Kim BS, Mooney DJ. Scaffolds for engineering smooth muscle under cyclic mechanical strain conditions. J Biomech Eng 2000; 122:210-215.
12. Zeltinger J, Landeen LK, Alexander HG, Kidd ID, Sibanda B. Development and characterization of tissue-engineered aortic valves. Tissue Eng 2001; 7:9-22.

13. Altman GH, Horan RL, Martin I, Farhadi J, Stark PR, Volloch V, Richmond JC, Vunjak-Novakovic G, Kaplan DL. Cell differentiation by mechanical stress. FASEB J 2002; 16:270-272.
14. Damsky C. Cell-cell and cell-extracellular matrix adhesion receptors. Ann N Y Acad Sci 2002; 961:154-155.
15. Zhang R, Yang H, Li M, Yao Q, Chen C. Acceleration of endothelial-like cell differentiation from CD14(+) monocytes in vitro. Exp Hematol 2005; 33:1554-1563.
16. Franceschi RT. Biological approaches to bone regeneration by gene therapy. J Dent Res 2005; 84:1093-1103.
17. Matsusaki M, Akashi M. Novel functional biodegradable polymer IV: pH-sensitive controlled release of fibroblast growth factor-2 from a poly(gamma-glutamic acid)-sulfonate matrix for tissue engineering. Biomacromolecules 2005; 6:3351-3356.
18. Huang Y, Siewe M, Madihally SV. Effect of spatial architecture on cellular colonization. Biotechnol Bioeng 2005; 93:64-75.
19. Hollister SJ. Porous scaffold design for tissue engineering. Nat Mater 2005; 4:518-524.
20. Mooney D, Hansen L, Vacanti J, Langer R, Farmer S, Ingber D. Switching from differentiation to growth in hepatocytes: control by extracellular matrix. J Cell Physiol 1992; 151:497-505.
21. Ingber DE, Dike L, Hansen L, Karp S, Liley H, Maniotis A, McNamee H, Mooney D, Plopper G, Sims J. Cellular tensegrity: exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis. Int Rev Cytol 1994; 150:173-224.
22. Ingber D. Mechanical signaling. Ann N Y Acad Sci 2002; 961:162-163.
23. Thomson RC, Mikos AG, Beahm E, Lemon JC, Satterfield WC, Aufdemorte TB, Miller MJ. Guided tissue fabrication from periosteum using preformed biodegradable polymer scaffolds. Biomaterials 1999; 20:2007-2018.
24. Bottaro DP, Liebmann-Vinson A, Heidaran MA. Molecular signaling in bioengineered tissue microenvironments. Ann N Y Acad Sci 2002; 961:143-153.

25. Schwartz Z, Boyan BD. Underlying mechanisms at the bone-biomaterial interface. J Cell Biochem 1994; 56:340-347.
26. Koller MR, Maher RJ, Manchel I, Oxender M, Smith AK. Alternatives to animal sera for human bone marrow cell expansion: human serum and serum-free media. J Hematother 1998; 7:413-423.
27. Han DK, Park KD, Hubbell JA, Kim YH. Surface characteristics and biocompatibility of lactide-based poly(ethylene glycol) scaffolds for tissue engineering. J Biomater Sci Polym Ed 1998; 9:667-680.
28. Korossis SA, Ingham E, Fisher J. Cardiac valve replacement: A bioengineering approach. Bio-Med Mat Eng 2000; 10:83-124.
29. Wilcox HE, Korossis SA, Watterson K, Kearney JN, Fisher J, Ingham E. Tissue engineering a living heart valve: Biocompatibility and recellularisation potential of an acellular porcine heart valve matrix. J Heart Valve Dis 2005; 14:228-237.
30. Booth C, Korossis SA, Wilcox HE, Watterson K, Kearney JN, Fisher J, Ingham E. Tissue engineering of cardiac valve prostheses I: Development and histological characterization of an accellular porcine scaffold. J Heart Valve Dis 2002; 11:457-462.
31. Cancedda R, Dozin B, Giannoni P, Quarto R. Tissue engineering and cell therapy of cartilage and bone. Matrix Biol 2003; 22:81-91.
32. Hutmacher DW, Vanscheidt W. Matrices for tissue-engineered skin. Drugs Today (Barc) 2002; 38:113-33.
33. Kasimir MT, Weigel G, Sharma J, Rieder E, Seebacher G, Wolner E, Simon P. The decellularized porcine heart valve matrix in tissue engineering: platelet adhesion and activation. Thromb Haemost 2005; 94:562-567.
34. Ruoslathi E. Integrins. J Clin Invest 1991; 87:1–5
35. Schwartz MA. Integrins, oncogenes, and anchorage independence. J Cell Biol 1997; 139:575-578.
36. Chien S, LI S, Shyy JYJ. Effects of mechanical forces on signal transduction and gene expression in endothelial cells. Hypertension 1998; 31:162–169.

37. Chen KD, Li YS, Kim M, Li S, Yuan S, Chien S, Shyy JY. Mechanotransduction in response to shear stress. Roles of receptor tyrosine kinases, integrins, and Shc. J Biol Chem 1999; 274:18393-18400.
38. Cartmell SH, Porter BD, Garcia AJ, Guldberg RE. Effects of medium perfusion rate on cell-seeded three-dimensional bone constructs in vitro. Tissue Eng 2003; 9:1197-1203.
39. Ignatius A, Blessing H, Liedert A, Schmidt C, Neidlinger-Wilke C, Kaspar D, Friemert B, Claes L. Tissue engineering of bone: effects of mechanical strain on osteoblastic cells in type I collagen matrices. Biomaterials 2005; 26:311-318.
40. Mauney JR, Sjostorm S, Blumberg J, Horan R, O'Leary JP, Vunjak-Novakovic G, Volloch V, Kaplan DL. Mechanical stimulation promotes osteogenic differentiation of human bone marrow stromal cells on 3-D partially demineralized bone scaffolds in vitro. Calcif Tissue Int 2004; 74(5):458-468.
41. Wiesmann HP, Joos U, Meyer U. Biological and biophysical principles in extracorporal bone tissue engineering. Part II. Int J Oral Maxillofac Surg 2004; 33(6):523-530.
42. Oluwole BO, Du W, Mills I, Sumpio BE. Gene regulation by mechanical forces. Endothelium 1997; 5:85-93.
43. Grinnell F. Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol 1994; 124:401-404.
44. Hung CT, LeRoux MA, Palmer GD, Chao PH, Lo S, Valhmu WB. Disparate aggrecan gene expression in chondrocytes subjected to hypotonic and hypertonic loading in 2D and 3D culture. Biorheology 2003; 40:61-72.
45. Schwachtgen JL, Houston P, Campbell C, Sukhatme V, Braddock M. Fluid shear stress activation of egr-1 transcription in cultured human endothelial and epithelial cells is mediated via the extracellular signal-related kinase 1/2 mitogen-activated protein kinase pathway. J Clin Invest 1998; 101:2540-2549.
46. Buschmann MD, Gluzband YA, Grodzinsky AJ, Hunziker EB. Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. J Cell Sci 1995; 108 (Pt 4):1497-1508.

47. Eschenhagen T, Fink C, Remmers U, Scholz H, Wattchow J, Weil J, Zimmermann W, Dohmen HH, Schafer H, Bishopric N, Wakatsuki T, Elson EL. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: a new heart muscle model system. FASEB J 1997; 11:683-694.
48. Young RG, Butler DL, Weber W, Caplan AI, Gordon SL, Fink DJ. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res 1998; 16:406-413.
49. Baskin L, Howard PS, Macarak E. Effect of physical forces on bladder smooth muscle and urothelium. J Urol 1993; 150(2 Pt 2):601-607.
50. Coplen DE, Howard PS, Duckett JW, Snyder HM, Macarak EJ. Cultured bladder cells and their response to mechanical strain. Adv Exp Med Biol 1995; 385:207-214; discussion 223-228.
51. Southgate J, Cross W, Eardley I, Thomas DF, Trejdosiewicz LK. Bladder reconstruction--from cells to materials. Proc Inst Mech Eng [H] 2003; 217:311-316.
52. Vunjak-Novakovic G, Martin I, Obradovic B, Treppo S, Grodzinsky AJ, Langer R, Freed LE. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. J Orthop Res 1999; 17:130-138.
53. Smith RL, Donlon BS, Gupta MK, Mohtai M, Das P, Carter DR, Cooke J, Gibbons G, Hutchinson N, Schurman DJ. Effects of fluid-induced shear on articular chondrocyte morphology and metabolism in vitro. J Orthop Res 1995; 13:824-831.
54. Vandenburgh HH, Hatfaludy S, Karlisch P, Shansky J. Mechanically induced alterations in cultured skeletal muscle growth. J Biomech 1991; 24 Suppl 1:91-99.
55. Matthews JB, Mitchell W, Stone MH, Fisher J, Ingham E. A novel three-dimensional tissue equivalent model to study the combined effects of cyclic mechanical strain and wear particles on the osteolytic potential of primary human macrophages in vitro. Proc Inst Mech Eng [H] 2001; 215:479-486.
56. Carver SE, Heath CA. Semi-continuous perfusion system for delivering intermittent physiological pressure to regenerating cartilage. Tissue Eng 1999; 5:1-11.

57. Schreiber RE, Dunkelman NS, Naughton G, Ratcliffe A. A method for tissue engineering of cartilage by cell seeding on bioresorbable scaffolds. Ann N Y Acad Sci 1999; 875:398-404.
58. Grande DA, Halberstadt C, Naughton G, Schwartz R, Manji R. Evaluation of matrix scaffolds for tissue engineering of articular cartilage grafts. J Biomed Mater Res 1997; 34:211-220.
59. Chromiak JA, Shansky J, Perrone C, Vandenburgh HH. Bioreactor perfusion system for the long-term maintenance of tissue-engineered skeletal muscle organoids. In Vitro Cell Dev Biol Anim 1998; 34:694-703.
60. Korossis SA, Wilcox H, Kearney J, Watterson K, Ingham E, Fisher J. Optimisation of culture conditions in a novel functional tissue engineering heart valve bioreactor. Proceedings of the Biomedical Engineering Society Annual Fall Meeting, Philadelphia, USA, October 13-16, 2004.
61. Niklason LE, Gao J, Abbott WM, Hirschi KK, Houser S, Marini R, Langer R. Functional arteries grown in vitro. Science 1999; 284:489-493.
62. Sodian R, Lemke T, Loebe M, Hoerstrup SP, Potapov EV, Hausmann H, Meyer R, Hetzer R. New pulsatile bioreactor for fabrication of tissue-engineered patches. J Biomed Mater Res 200; 58:401-405.
63. Schoenwaelder SM, Burridge K. Bidirectional signaling between the cytoskeleton and integrins. Curr Opin Cell Biol 1999; 11:274-286.